

# SEXUAL DIMORPHISM IN *HAPLOMITRIUM INTERMEDIUM*

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[Accepted for publication 20th September 1972]

## Synopsis

Sexual dimorphism in *Haplomitrium intermedium* G. K. Berrie has been investigated and confirmed and found to be correlated with a sex chromosome mechanism.

## INTRODUCTION

The order Calobryales is known to be represented only by *Haplomitrium intermedium* G. K. Berrie in Australia. It was noted by Berrie (1962) that there is an apparent dimorphism between the female and male gametophytes; and later references to the genus (Schuster, 1967, 1971) suggest that other species have dimorphic gametophytes. Herein an attempt has been made to analyse the dimorphism in *H. intermedium*. It was done by (i) measurement of the morphological differences and (ii) a study of the cytology.

## MORPHOLOGY

A random sample of the plants at the type locality was collected and maintained in the laboratory. From these plants, a further random sample of 25 female and 25 male plants was selected for quantitative measurement. In order to be certain (a) that the correct sex was selected, and (b) that there were no errors due to stage of maturity, only plants bearing terminal archegonia and antheridia, respectively, were selected. For each plant:

- (i) Leaves. The top 10 leaves were dissected off serially from the tip and the following measurements taken: (a) *length* and (b) *width*, both measured at 40 times magnification. In analysing the results, it was noted that the measurements for leaf length and width fell into two populations. This was due to the larger "perianth" leaves surrounding the archegonia and antheridia. Hence, "perianth" leaves have been separated from the "lower" leaves for the analysis. (c) *Cell area* at the tip of each leaf. To do this, a square was inserted in the eyepiece of the microscope and the area of the resultant field of view measured. Then the number of complete and incomplete cells was scored and the mean cell area calculated by use of the formula

$$\frac{\text{Total area}}{\text{Number of complete cells} + \frac{1}{2} \text{ number of incomplete cells}} = \text{Mean cell area}$$

- (ii) Stems. A transverse section of the stem was cut and the diameter measured at 100 times magnification. A further sample of 30 female and 30 male plants was taken for this measurement.

## RESULTS

The means for the leaf and stem measurements were calculated and from these values an *F* test was carried out on each of stem diameter, cell area, leaf length ("perianth" and "lower"), leaf width ("perianth" and "lower") and leaf length/width ratio (Table 1 (ii)), to test if the sample variances are

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TABLE 1

Measure under Analysis	Mean $\bar{X}$	Standard Deviation (SD)	Standard Error (SE)	(i) $t$ Value	Prob. of Mean Independ.	(ii) $F$ Value	Significance Level		Conclusion	
							5%	1%	(i)	(ii)
Stem diameter (mm) ..	♀ 0.60	0.12	0.02	21.4	0.001	55.57	3.94	6.90	Significant difference	Significant difference
	♂ 0.43	0.10	0.01							
Cell area $x \times 10^2$ (sq $\mu$ )	♀ 310.30	79.70	4.60	1.4	0.15	3.44	4.04	7.19	No significant difference	No significant difference
	♂ 306.10	60.30	3.40							
Leaf length, lower (mm)	♀ 1.71	0.36	0.03	26.4	0.001	74.17	4.04	7.19	Significant difference	Significant difference
	♂ 1.12	0.30	0.02							
Leaf length, perianth (mm)	♀ 2.22	0.48	0.04	37.5	0.001	161.89	4.04	7.19	Significant difference	Significant difference
	♂ 1.23	0.29	0.03							
Leaf width, lower (mm)	♀ 1.16	0.45	0.04	34.7	0.001	30.46	4.04	7.19	Significant difference	Significant difference
	♂ 0.78	0.32	0.02							
Leaf width, perianth (mm)	♀ 1.34	0.51	0.04	16.3	0.001	19.05	4.04	7.19	Significant difference	Significant difference
	♂ 0.91	0.35	0.03							
Leaf length/width, lower	♀ 1.54	0.60	0.05	3.8	0.01	1.35	4.04	7.19	Significant difference	No significant difference
	♂ 1.39	0.46	0.03							
Leaf length/width perianth	♀ 1.67	0.65	0.06	6.0	0.01	9.42	4.04	7.19	Significant difference	Significant difference
	♂ 1.36	0.37	0.03							

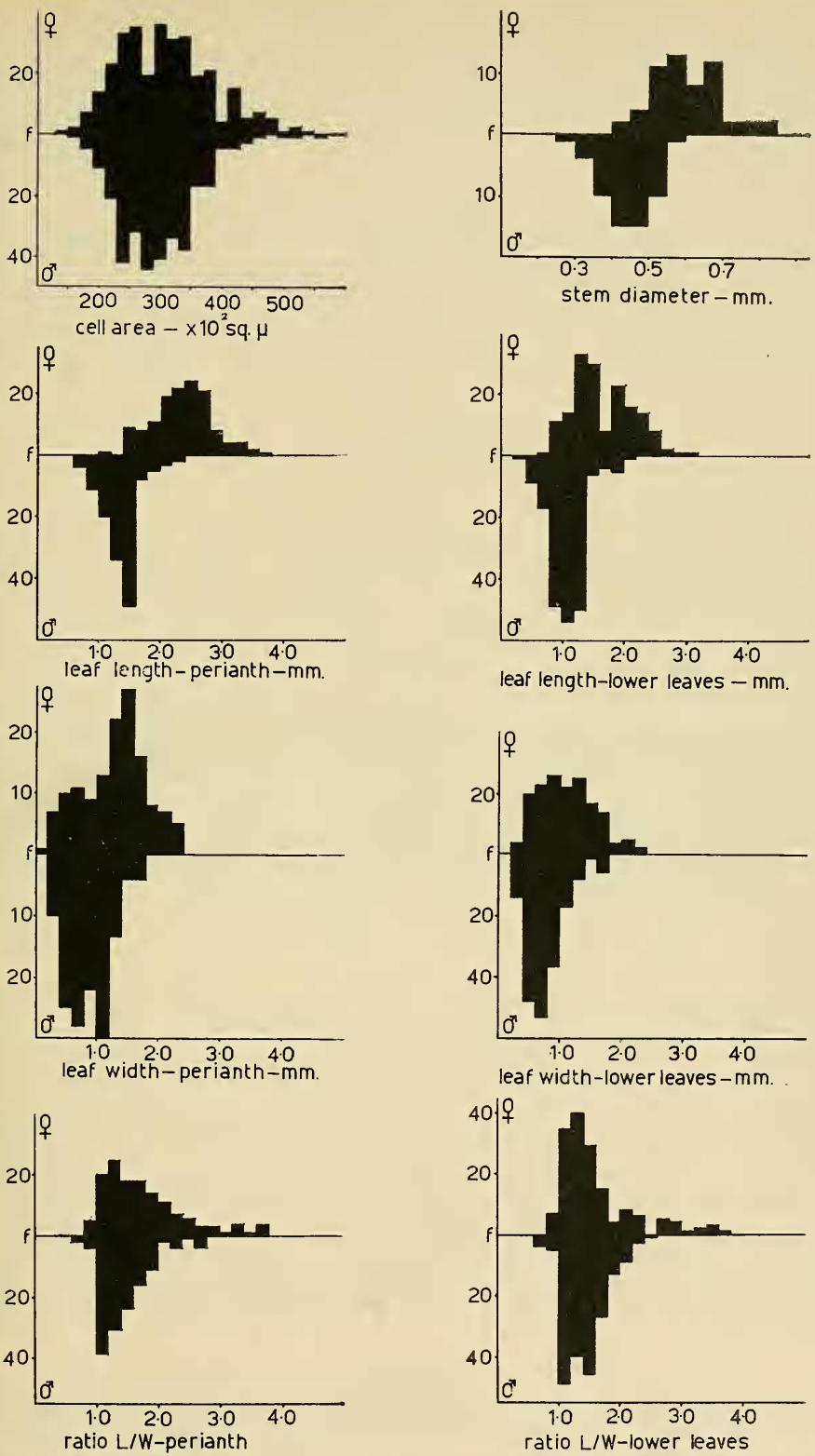


Fig. 1. Frequency distributions for characters analyse  $\bar{dx}$  = mean, sd = standard deviation, and se = standard error.

significantly different. In addition, the values for stem diameter, cell area, leaf length, leaf width and leaf length/width ratio for female and male plants have been presented in frequency distributions (Fig. 1). The *t* test was applied to these to test if the means for male and female characters are significantly different (Table 1 (i)).

#### INTERPRETATION

From Table 1, it can be seen that there are significant differences in the morphology of the female and male gametophytes of *Haplomitrium intermedium*. The female gametophytes have larger "perianth" and "lower" leaves, and have thicker stems than do the male gametophytes. However, it does appear that cell area and leaf length/width ratio are inherent properties of the species and are independent of sexual morphology.

#### CYTOLOGY

An attempt was made to investigate if the marked sexual dimorphism is reflected in the cytology.

Stolon tips, shoot tips and developing sporophytes were selected from material collected at the type locality and fixed in 3 : 1 Absolute Alcohol : Glacial Acetic Acid, for 12 hours at room temperature. These were transferred to Absolute Alcohol and stored at 0° C. Squash preparations were made and stained in Aceto-orcein (Darlington and LaCour, 1960). It was found to be necessary to macerate the gametophyte material. This was done by pretreating the tips in snail cytase for 45–60 minutes at room temperature.

From mitotic studies when comparing the female and male chromosome complements it was found that there is one chromosome which does not have an obvious homologue in the other's complement. This chromosome in the female complement displays positive heteropycnosis, having a heterochromatic knob at one end (Fig. 2). The odd chromosome in the male complement does not show any convincing evidence of heteropycnosis (Fig. 2). The chromosome No. 3 in the female complement is associated with nucleolar organization (NO). The nucleolar organizer is unconfirmed in the male complement. The longest chromosome (No. 1) and the shortest chromosome (No. 8) do not show any evidence of heteropycnosis in either complement, however they probably correspond to the M (macro-) and m (micro-) chromosome (Berrie, 1959).

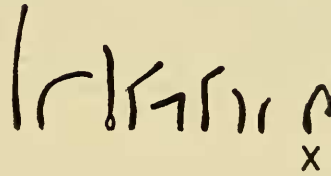
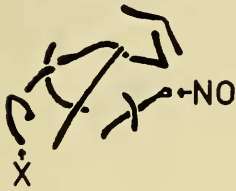
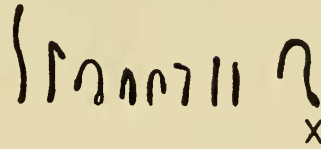
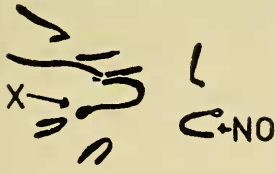
In meiosis it was observed that Prophase I is not clear until late Diplotene. At Diakinesis there was found to be a mean of 18·9 chiasmata per cell and 2·1 chiasmata per bivalent. At Metaphase I the chiasmata are almost completely terminalized. However, it was noted that terminalization is completed earlier in one bivalent, and this bivalent tends therefore to enter Anaphase I precociously (Fig. 3). It was also observed that this bivalent is negatively heteropycnotic and has homologues of unequal length. This bivalent is obviously composed of the two unequal homologues observed in the mitotic karyotype studies. It seems reasonable to assume that this is a sex chromosome mechanism. The longer chromosome is always associated with the female complement and is the X chromosome, and the shorter chromosome is always associated with the male complement and is the Y chromosome. After Anaphase I, the chromosomes pass into a temporary interphase before starting a rapid second division. The final product of the division is two spores with  $n=8+X$  and two spores with  $n=8+Y$ .

#### SUMMARY

From these studies, it can be seen that there is dimorphism between the female and male gametophytes of *Haplomitrium intermedium*. The male

MITOSIS  
FEMALE

KARYOTYPE INTERPRETATION



MALE

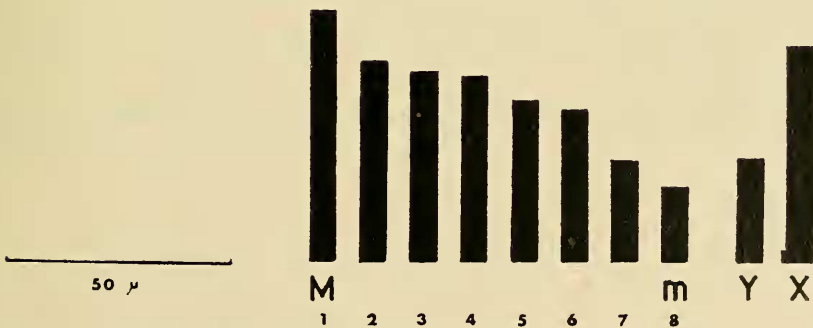
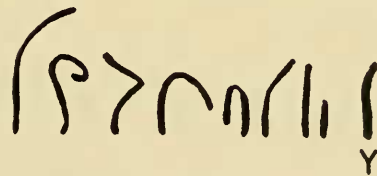
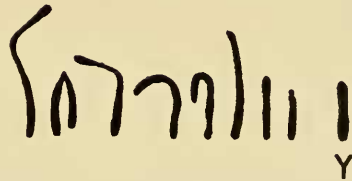
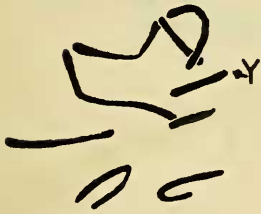


Fig. 2. Chromosome karyotype interpretation. M=macrochromosome, m=microchromosome, NO=nucleolar organizer, X & Y=sex chromosomes.

gametophytes have thin stems, small leaves and a less marked perianth relative to the female gametophytes. Moreover, it can be seen that this dimorphism is associated with a sex chromosome mechanism.

## MEIOSIS — METAPHASE I



Fig. 3. Meiosis—metaphase I. X-Y=sex chromosome bivalent.

### ACKNOWLEDGEMENTS

I would like to thank Dr. G. K. Berrie for introducing me to Bryology, and for his encouragement with this project.

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